The response of seedlings of two dipterocarp species to nutrient additions and ectomycorrhizal infection

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Abstract
An experiment was conducted to investigate the effect of ectomycorrhizal infection on growth and nutrient uptake, especially of P and K of dipterocarp seedlings. Hopea helferi (Dyer) Blanco and Hopea odorata Roxb. seedlings were grown in a sandy loam soil given a basal dressing. Nutrient treatments were unamended soil (NIL), amended soil with the addition of P and K (F), amended soil without P but with K (-P), amended soil without K but with P (-K), amended soil without P or K addition (-PK). Seedlings grown in the amended soil treatments showed foliar symptoms suggestive of calcium deficiency. Ectomycorrhizal infection appeared to improve shoot Ca concentration and relieved the foliar symptoms. Ectomycorrhizal infection in H. odorata plants increased shoot P concentration and increased shoot and total dry weight to the same or greater extent than those of uninfected plants growing on P amended soil. H. helferi showed a positive response to ectomycorrhizal infection in shoot, root and total dry weight in all nutrient treatments but no response to the nutrient treatments themselves.

Introduction
In Malaysia tropical rainforests are mostly found on well drained ultisols and oxisols of low mineral content (Burnham in Whitmore, 1988). These soils are susceptible to nutrient loss upon removal of the forest cover.

There is currently much interest, throughout Southeast Asia, in establishing plantations of indigenous forest trees, especially of dipterocarps, which are the most important family of commercial timber trees in Malaysia. This has increased interest in dipterocarp mycorrhizas (Lee, 1990) since they may be important for successful seedling establishment and for enhanced uptake of nutrients. Although much has been documented about the effects of ectomycorrhizal infection on plant growth and uptake of nutrients, especially phosphorus, little of this information concerns tropical tree species (Alexander, 1989; Harley and Smith, 1983) and even less is known about dipterocarps (Lee, 1990).

Phosphorus (P) is thought to be the limiting nutrient in most tropical forests (Unesco/UNEP/FAO, 1978; Vitousek, 1984). However, the basal area of the common dipterocarp Shorea leprosula (Miq.) in two forest reserves in Malaysia, was positively correlated with foliar potassium (K), and there was a highly significant correlation between foliar K and total soil K (Amir Husni and Miller, 1990). Baillie et al. (1987) working in mixed dipterocarp forest in Sarawak found a highly significant negative relationship between soil magnesium (Mg) status and occurrence of some dipterocarp species. They suggested that mycorrhizal dipterocarps in the mixed dipterocarp forest depended on magnesium for efficient uptake of P from soils of low P levels.

In three independent experiments Turner and his coworkers (1993) found that application of fertilizers had no effect on the growth of potted or wild dipterocarp seedlings greater than eight months old. In one experiment fertilisation increased ectomycorrhizal infection, but seedling size and infection were only correlated in unfertilised seedlings. Becker (1983) and Lee and Lim (1989) also found correlations between dipte-
rocarp seedling growth and ectomycorrhizal infection. In the latter case infection was also correlated with foliar P concentration.

In research on temperate species the role of ectomycorrhizas has been clarified by comparing the growth of mycorrhizal and non-mycorrhizal plants under various nutrient regimes. One pot experiment adopting this approach is reported here. Phosphorus and potassium were the elements chosen for investigation.

Because many dipterocarps fruit infrequently and because the seed of many species is recalcitrant (Tompsett, 1991), the choice of experimental species was determined by seed availability. This experiment was conducted with Hopea helferi (Dyer) Blanco and Hopea odorata Roxb., members of the scaly barked Hopeas in Group IV of section Euhopea Brandis (Symington, 1974).

Materials and methods

Soil preparation

Sandy loam obtained from a 40 year-old plantation of Dryobalanops aromatica and Dipterocarpus spp. was mixed with sand in the proportion of 7:3, steam sterilised twice at 90°C for 1h over two days, and then oven dried at 70°C. One portion of the soil was set aside (NIL) while nutrients except P and K were applied as laboratory grade chemicals to the remainder at the following rates kg⁻¹ soil according to Bougher et al. (1990): 29 mg Ca (Ca(H₂PO₄)H₂O), 18 mg N (NH₄NO₃), 4.2 mg Mn (MnCl₂ 4H₂O), 3.3 mg Mg (MgSO₄·7H₂O), 2.1 mg Zn (ZnSO₄·7H₂O), 2.1 mg Cu (CuSO₄·5H₂O), 0.12 mg B (H₃BO₃), 0.08 mg Co (CoSO₄·7H₂O), 0.25 mg Mo (Na₂MoO₄·2H₂O) and 6.0 mg Fe. The nutrient amended soil was thoroughly mixed and dried at 35°C.

Test plants and fungal inoculum

Seeds of H. helferi and H. odorata were collected from trees growing in the FRIM compound in mid-November 1991 and germinated in washed sand. Four weeks later single fully developed non-mycorrhizal seedlings were transplanted into black polythene bags containing 500g of sterile, nutrient amended soil or sterile, unamended soil (NIL), one seedling per bag. Root inoculum consisting of 3.0g fresh or autoclaved chopped H. odorata roots obtained from one year-old seedlings in the FRIM nursery was incorporated into the top five cm of each 500g bag of soil at the time of transplanting.

The transplanted seedlings were kept in the laboratory and watered daily with distilled water for one week before being transferred into a shade house in late December 1991. The photon flux density in the shade house varied from 60 μmol m⁻² s⁻¹ at 0900 h to a maximum of 395 μmol m⁻² s⁻¹ at 1200 h.

Nutrient treatments

The nutrient treatments were (I) unamended sterile soil (NIL), (II) amended soil with the addition of P and K at the rates of 40 mg powdered Ca(H₂PO₄)H₂O and 50 mg KCl kg⁻¹ soil (F), (III) amended soil minus P but with K added at the above rate (-P), (IV) amended soil minus K but with P added at the above rate (-K), and (V) amended soil without P or K additions (-PK).

There were 20 replicates for each nutrient treatment, 10 with mycorrhizal inoculum, and 10 without, for each species. At three weekly intervals, 9 mg N was added as NH₄NO₃ in 12.5 mL solution to each bag in all except the NIL treatment.

Ammonium chloride-extractable ammonium and nitrate nitrogen, Bray 2 phosphorus (Bray and Kurtz, 1945) and ammonium acetate-exchangeable potassium, calcium and magnesium were determined (Table 1).

Initially the unamended soil had high levels of ammonium-N (72 μg g⁻¹) and available P (20 μg g⁻¹) but low levels of nitrate-N (3 μg g⁻¹). P amended soils contained 70–90 μg g⁻¹ P. Unamended soils contained 0.03–0.05 meq K 100 g⁻¹; this rose after amendment to 0.15-0.23 meq 100 g⁻¹.

At the end of the experiment ammonium-N was undetectable in most treatments but nitrate-N was 26–74 μg g⁻¹. Available P had dropped to approximately 10 μg g⁻¹ in unamended and approximately 29 μg g⁻¹ in P-amended soil. The equivalent values for K were 0.06 and 0.17 meq 100 g⁻¹ respectively. Soil amendment did not reduce the amount of exchangeable calcium in the soil.

Plant harvest

Seedling height, number of leaves and nodes were measured at 14-day intervals and the plants were harvested 100 days after planting. Individual shoot and root dry weights (80°C) were measured. Roots were examined for ectomycorrhizal infection at 40 to 500 times magnification prior to drying.